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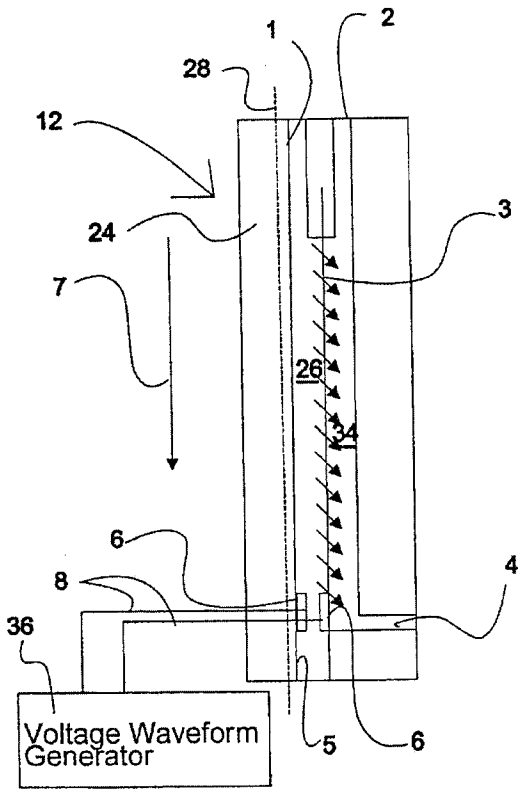
(57) Abstract

An object of the invention is to provide a tangential flow, cell concentration and fusion apparatus (12) which includes a transparent housing (24) and a first input channel (1) contained in the housing (24). In the housing (24), a concentration chamber (26) includes a proximal end connected to the first input channel (1) and extends along a longitudinal axis (28). A first output channel (5) is connected to a distal end of the concentration chamber (26). A filter (3) serves as a wall of the concentration chamber (26), is located between the first input channel (1) and the first output channel (5), and extends in a direction parallel to the longitudinal axis (28).



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TANGENTIAL FLOW, CELL CONCENTRATION AND FUSION APPARATUS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority based upon copending United States Provisional Application Serial No. 60/125,583, filed 22 March 1999.

Technical Field

The present invention relates generally to methods and apparatus for fusing biological cells together. More specifically, the present invention provides methods and apparatus for fusing biological cells using the application of electrical fields to the biological cells to be fused together.

Background Art

Cell fusion is useful for a variety of research and clinical applications. The most common application for cell fusion is the fusion of a B cell and a myeloma cell to make a hybridoma. Hybridomas are used for the production of monoclonal antibodies. Any two cells from closely related species can potentially be fused for the purpose of taking advantage of characteristics from each fusion partner.

There are many possible clinical uses for fused cells. The cell fusion that is currently being explored in many laboratories is fusion between dendritic cells and leukemia cells. This cell fusion is done for the purpose of inducing an immune response to the leukemia cells. The advantage of this approach is that cancer antigens do not have to be known for this process to work.

A problem to date with on-going research is that the number of desirable fused-cell products generated by existing technology is relatively small (low efficiency). Thus, a cell fusion technology that is more efficient would be immediately and widely accepted.

An important number for any cell fusion system is the percentage of cells that are fused. The probability of getting fusion products in a system which has the

objective of fusing two and only two cell types, that have equal cell populations, are:

Cell Type 1 to Cell Type 1	25%	of no use
Cell Type 1 to Cell Type 2	25%	desired
Cell Type 2 to Cell Type 1	25%	desired
Cell Type 2 to Cell Type 2	25%	of no use

Thus the maximum possible percentage of desired cell products, which is Cell Type 1 fused with Cell Type 2, is 50%.

Currently, there are two classes of methods for fusing cells: chemical and electrical.

The chemical method usually involves concentrating cells in the presence of polyethylene glycol. This method is toxic to cells and the maximum yields of desirably fused cells are approximately only 0.1 %.

The electrical method for fusing cells requires first contact between the cell membranes of two or more cells, and then the application of a high voltage electrical pulse. The high voltage electrical pulse induces cellular pores (openings) which lead to cell fusion in the touching cells. The contact between the cell membranes prior to induction of cell fusion is a critical part of the process. The method of bringing the cells into contact highly influences the percent of different-cell-type fused cell pairs (desired), the percent of same-cell-type cells fused (not desired), and the viability of the cells fused. The method of applying the electrical pulses also has an effect on the efficiency. To date ordinary pulses have been used, exponential decay pulses (see Sowers, A.E., 1986, J. Cell Biology 102:1358-1362; Sowers, A.E., 1988, Biophys. J. 54: 619-626; and Sowers, A.E. 1989, Electroporation and Electrofusion, Plenum, 229-256), and squarewave pulses (see Heller, R. et. al., 1990, Biochm Biophys Acta, 1024: 185-188; Tessie, J., et. al., 1984, Biochm Biophys Acta, 775: 446-448; Tessie, J. et. al., 1982, Science, 216: 637-

538; and Tessie, J., et. al., 1986, Biochm Biophys Res, 140: 258-266).

It expected that a wide variety of suitable waveforms can be selected and applied by using the programmable pulse generator in the PulseAgile (Registered in U. S. Patent and Trademark Office), Model PA-4000 Electroporation System made by Cyto Pulse Sciences, Inc., P. O. Box 609, Columbia, MD 21045. It is noted that the Model PA-4000 delivers rectangular waves of various amplitudes (voltages), width, and intervals. It is expected that use of the PulseAgile System may improve efficiency.

Bringing different-cell-type cells into contact is a key factor in an efficient cell fusion process, and there are four classes of known methods to bring such cells into contact: adherence, chemical, electrical and mechanical.

With the adherence method, cells are brought into contact by cell culture confluence (see Tessie, J., et. al., 1984, Biochm Biophys Acta, 775: 446-448; Tessie, J. et. al., 1982, Science, 216: 637-538; Finaz, C. et. al., 1984, Exp Cell Res, 150: 477-482; and Sukharev, S., 1990, Biochm Biophys Acta, 1034: 125-131). A problem with the adherence approach is that the cell type must be adherent, that is lay on the bottom of a container and not float in suspension.

With the chemical method of bringing cells into contact, antibodies and chemical linkers on unlike cells are designed to increase the probability of having unlike cells fuse together rather than like cells fusing. This method can be used simultaneously with the other methods. A problem associated with this method is that other molecules, aside from the antibodies and the chemical linkers, can also attach to the surfaces of cells, and these other molecules could interfere with the desired technique (see Lo, M., 1984, Nature 310-792-794; Hewish, D., 1989, J. Immunology Methods, 120: 285-289; Wojchowski,

D., 1986, J. Immunology Methods, 90: 173-277; and Bakker, S., 1993, Biophy. J. 65: 568-572).

A well-known electrical method for bringing cells into contact is called dielectrophoresis, which was developed by Zimmerman in 1982 (see Zimmerman, U., 1982, Biochm Biophys Acta, 694: 227-277). Dielectrophoresis is the induction of dipoles in cells through the application of a low voltage potential. This is usually applied as a very high frequency sine wave, but direct current can also be used. The dipoles in the cells cause the cells to line-up in a chain called a pearl chain. A brief high voltage electrical pulse is applied to the cells while they are lined up.

With the above-mentioned Model PA-4000 Electroporation System made by Cyto Pulse Sciences, Inc., P. O. Box 609, Columbia, MD 21045, a dielectrophoresis option is provided. That unit has been in the field since May 1997. A problem with the dielectrophoresis technique is that the time the AC or DC field is on may be seconds to minutes. Even at a low voltage, this length of time causes heating of the media in which the cells are are placed, resulting in cell damage. Another problem with this technique is the two types of cells to be fused must be approximately the same size.

Two mechanical methods of bringing about cell contact are considered: centrifugation; and vacuum concentration.

With respect to centrifugation, there are three approaches:

1. The cells are pulsed with electricity and then placed in the centrifuge and packed together. However, the centrifugal force damages the cells that have already been porated (see Tessie, J., et. al., 1986, Biochm Biophys Res, 140: 258-266).

2. The high voltage pulses are delivered during the centrifugation after the cells have packed into a pellet. This method is very efficient but it tends to induce

multiple cells (more than two) to fuse, and the cell viability is poor (see Abidor, I., 1993, Biochm Biophys Acta., 1152: 207-218).

3. The high voltage pulses are delivered after centrifugation.

Turning to vacuum concentration, a vacuum technique has been developed by Mark Jaroszeski, Richard Gilbert, and Richard Heller at the University of South Florida (see Jaroszeski, M., et. al., 1994, Biophy. J. 67: 1574-1581). In this technique, cells were placed on two identical assemblies which consisted of a stainless steel tube on top of which was a stainless steel screen about one centimeter in diameter. A porous filter was on top of the screen. The hole diameter in the porous filter was small enough to let the water solution through but not the cells. A vacuum was applied at the end of each stainless steel tube, and the vacuum drew out the water and left the cells on the filter. Water flow through the porous filter was in a direction perpendicular to the surface of the porous filter. The two faces were then placed together, and the stainless steel screens were pulsed. The pulsing was conducted normal to the surface of the porous filter. This device produced cell fusion efficiencies of up to 10%.

The University of South Florida group has also published an assay to measure the number of cells fused (see Jaroszeski, M., et. al., 1994, Analytical Biochem., 216: 271-275). The assay is a clever technique that dyes Cell Type 1 red and Cell Type 2 green. The fused cells then become yellow and can be counted by a flow cytometer.

Of the methods mentioned above, the methods in which the biological cells are concentrated prior to electrofusion are most desirable. With respect to the vacuum concentration methods developed by the University of South Florida group, it is noted that the liquid flow flows in a direction perpendicular to the surface of the porous filter. In this respect, the liquid flow is

obstructed by the porous filter. To lessen obstruction to liquid flow, it would be desirable if a cell fusion apparatus were provided which employs a concentrating porous filter which is not oriented perpendicular to the liquid flow through the apparatus.

Aside from the methods of the University of South Florida group, a body of art exists relating to other cell fusion apparatuses. In a number of these other cell fusion apparatuses, there is a substantially unobstructed path of liquid flow through the apparatus. However, these other cell fusion apparatuses lack an important feature. They do not provide efficient concentration of the cells prior to electrofusion. These cell fusion apparatuses do not have porous filters at which a concentration of cells occur. Such non-concentrating-filter-containing cell fusion apparatuses are represented by the following U. S. patents: 4,441,972, 4,476,004, 4,800,163, and 4,832,814. Although it is desirable to have cell fusion apparatuses which have unobstructed flow through of liquid, it is undesirable not to have cell concentration. In this respect, it would be desirable if a cell fusion apparatus provides both unobstructed liquid flow and concentration of biological cells that are to undergo electrofusion.

There is yet another body of prior art devices which disclose tangential flow filtration without the use of electrical fields. Such devices are used to carry out conventional concentration and washing steps. However, these prior art tangential flow filtration devices do not employ electrical fields to treat biological cells for electroporation or electrofusion purposes. It would be desirable, however, if an electroporation or electrofusion apparatus were provided which employs tangential flow filtration.

Still other features would be desirable in a cell fusion apparatus. For example, it would be desirable if a cell fusion apparatus could be contained in a sealed, sterile system. In this respect, it would be desirable

if a cell fusion apparatus were modular in form that could be used, disposed of, and replaced in a simple and easy manner. For a modular cell fusion apparatus, it would be desirable if a cell fusion module could be placed in a module manipulator that interacts with and operates a cell fusion module from outside the module without entering the module so that the sterility inside the module is not disturbed.

Thus, while the foregoing body of prior art indicates it to be well known to use cell fusion apparatuses, the prior art described above does not teach or suggest a cell fusion apparatus which has the following combination of desirable features: (1) employs a concentrating porous filter which is not oriented perpendicular to the direction of liquid flow through the apparatus; (2) provides both unobstructed liquid flow and concentration of biological cells that are to undergo electrofusion; (3) provides an electroporation or electrofusion apparatus which employs tangential flow filtration; (4) can be contained in a sealed, sterile system; (5) can be modular in form and can be used, disposed of, and replaced in a simple and easy manner; and (6) can employ a programmable module manipulator that interacts with and operates a cell fusion module from outside the module without entering the module so that the sterility inside the module is not disturbed. The foregoing desired characteristics are provided by the unique tangential flow, cell concentration and fusion apparatus of the present invention as will be made apparent from the following description thereof. Other advantages of the present invention over the prior art also will be rendered evident.

Disclosure of Invention

In accordance with one aspect of the invention, a cell concentration and fusion apparatus includes a housing. A first input channel is contained in the

housing. A concentration chamber includes a proximal end connected to the first input channel and is contained in the housing. The concentration chamber extends along a longitudinal axis through the housing. A first output channel is contained in the housing and is connected to a distal end of the concentration chamber. A filter serves as a wall of the concentration chamber. The filter is located between the first input channel and the first output channel. The filter extends in a direction parallel to the longitudinal axis. An effluent chamber is contained in the housing. The filter serves as a wall of the effluent chamber. A second output channel is connected to the effluent chamber. Electrodes are supported by the housing, and the electrodes are located between the first input channel and the first output channel. The housing is made of transparent material. The electrodes are located adjacent to the first output channel, and, in one configuration, the electrodes are oriented parallel to the longitudinal axis.

The filter is a porous membrane and has a porosity which prevents biological cells from passing through but permits liquids to pass through.

A second input channel is contained in the housing, and the second input channel is connected to the effluent chamber. The second input channel serves as a vent channel.

In accordance with another embodiment of the invention, a cell mixture bladder is connected to the first input channel. A liquid bladder is connected to the first input channel. A fused cell bladder is connected to the first output channel, and an exhaust bladder is connected to the second output channel. A first valve is located between the cell mixture bladder and the first input channel. A second valve is located between the liquid bladder and the first input channel, and a third valve is located between the fused cell bladder and the first output channel.

An input port is connected to the cell mixture bladder. An output port is connected to the fused cell bladder, and an electrical connection jack is electrically connected to the wires. The input port and the output port can be made from self-sealing elastomeric material, which is suitable for receiving a hypodermic needle.

A bag envelopes the cell concentration and fusion apparatus, the cell mixture bladder, the liquid bladder, the fused cell bladder, the exhaust bladder, and the wires for providing a cell fusion module. The bag leaves access to the input port, the output port, and the electrical connection jack. The bag is hermetically sealed. The interior of the cell fusion module is maintained in a sterile status by the bag. The bag is made from flexible material.

In accordance with another embodiment of the invention, a module manipulator unit, which can be programmable, is provided for receiving and operating the cell fusion module. The module manipulator unit includes a manipulator housing. A first bladder operation assembly is supported by the manipulator housing for operating the cell mixture bladder. A second bladder operation assembly is supported by the manipulator housing for operating the liquid bladder, and a bladder assemblies controller is provided for controlling the first bladder operation assembly and the second bladder operation assembly.

A third bladder operation assembly can be supported by the manipulator housing for operating the fused cell bladder, and a fourth bladder operation assembly, supported by the manipulator housing, can be provided for operating the exhaust bladder. The third bladder operation assembly and the fourth bladder operation assembly are also controlled by the bladder assemblies controller.

Each of the bladder operation assemblies can include a respective shaft drive unit driven by the bladder assemblies controller. A respective shaft is driven by

the shaft drive unit, and a respective ram is moved by the shaft.

In accordance with another embodiment of the invention, the cell concentration and fusion apparatus includes a base block and a front block that is supported by the base block. The front block includes a milled front slot, a vent channel, front fastener-reception channels, and a drain channel.

A back block is supported by the base block and includes an input channel, a transverse hole in communication with the input channel, a milled back slot in communication with the transverse hole, an electrode, an output channel in communication with the back slot, a pair of electrical jacks connected to the electrode, and back fastener-reception channels.

A gasket is located adjacent to the front block between the front block and the back block. The gasket includes gasket fastener-reception channels and a filter access channel. A filter is located between the gasket and the back block.

Fasteners are received in the back fastener-reception channels, the gasket fastener-reception channels, and the front fastener-reception channels when the back fastener-reception channels, the gasket fastener-reception channels, and the front fastener-reception channels are placed in registration.

In accordance with another embodiment of the invention, the electrodes are supported by the housing and are oriented perpendicular to the longitudinal axis.

In accordance with another embodiment of the invention, a method is provided for concentrating and electrically treating biological cells. The method includes the steps of:

obtaining a mixture of the biological cells in a liquid carrier,

moving a portion of the cell/liquid mixture into a solid/liquid separation chamber in a first direction towards an electrical treatment region,

separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells,

collecting the biological cells that have been concentrated and treated with the electrical field.

The method of the invention includes the step of applying an electrical field to the concentrated biological cells. In this respect, the method of the invention can include applying an electrical field to bring about dielectrophoresis in the concentrated biological cells, can include applying an electrical field to bring about cell fusion in the concentrated biological cells, and can include applying an electrical field to bring about dielectrophoresis in the fused cells to result in post-fusion maturation of the fused cells.

As stated above, the electrical field that is applied to the concentrated cells can bring about cell fusion. Alternatively, the electrical field that is applied to the concentrated cells can bring about electroporation.

In accordance with another embodiment of the invention, in the context of employing the cell fusion module and the module manipulator unit, a method of concentrating and electrically treating biological cells includes the steps of:

obtaining a cell fusion module which includes a cell concentration and fusion apparatus,

placing the cell fusion module in a programmable module manipulator unit,

obtaining a mixture of the biological cells in a liquid carrier,

introducing a portion of the cell/liquid mixture into the cell concentration and fusion apparatus, under control of the module manipulator unit,

moving a portion of the cell/liquid mixture into a solid/liquid separation chamber in the cell concentration and fusion apparatus, in a first direction, towards an electrical treatment region in the cell concentration and fusion apparatus,

separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells inside the cell concentration and fusion apparatus, and

moving the concentrated electrial-field-treated cells out from the cell concentration and fusion apparatus.

Then, the electrial-field-treated cells can be moved either to a collection region outside the cell fusion module or to a collection region inside the cell fusion module. If moved to a collection region inside the cell fusion module, then, the electrial-field-treated cells are moved to a location outside the cell fusion module.

Additionally, the cell fusion module can be considered disposable and can be disposed of in its entirety after the fused cells have been collected.

The above brief description sets forth rather broadly the more important features of the present

invention in order that the detailed description thereof that follows may be better understood, and in order that the present contributions to the art may be better appreciated. There are, of course, additional features of the invention that will be described hereinafter and which will be for the subject matter of the claims appended hereto.

In this respect, before explaining a number of the embodiments of the invention in detail, it is understood that the invention is not limited in its application to the details of the construction and to the arrangements of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced and carried out in various ways. Also, it is to be understood, that the phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting.

As such, those skilled in the art will appreciate that the conception, upon which disclosure is based, may readily be utilized as a basis for designing other structures, methods, and systems for carrying out the several purposes of the present invention. It is important, therefore, that the claims be regarded as including such equivalent constructions insofar as they do not depart from the spirit and scope of the present invention.

It is therefore an object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus which employs a concentrating porous filter which is not oriented perpendicular to the direction of liquid flow through the apparatus.

Still another object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus that provides both

unobstructed liquid flow and concentration of biological cells that are to undergo electrofusion.

Yet another object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus which provides an electroporation or electrofusion apparatus which employs tangential flow filtration.

Even another object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus that can be contained in a sealed, sterile system.

Still a further object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus which can be modular in form and can be used, disposed of, and replaced in a simple and easy manner.

Yet another object of the present invention is to provide a new and improved tangential flow, cell concentration and fusion apparatus that can employ a module manipulator that interacts with and operates a cell fusion module from outside the module without entering the module so that the sterility inside the module is not disturbed.

These together with still other objects of the invention, along with the various features of novelty which characterize the invention, are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its operating advantages and the specific objects attained by its uses, reference should be had to the accompanying drawings and descriptive matter in which there are illustrated preferred embodiments of the invention.

Brief Description of Drawings

The invention will be better understood and the above objects as well as objects other than those set

forth above will become more apparent after a study of the following detailed description thereof. Such description makes reference to the annexed drawing wherein:

Fig. 1 is a cross-sectional view of a first embodiment of the tangential flow, cell concentration and fusion apparatus of the invention which employs electrodes oriented parallel to the liquid flow path.

Fig. 2 is a schematic diagram of a second embodiment of the invention in which the embodiment of the invention shown in Fig. 1 is contained in a sterile cell fusion module.

Fig. 3 is a schematic diagram of a third embodiment of the invention in which the cell fusion module shown in Fig. 2 is placed in a module manipulator unit.

Fig. 4 is a schematic close-up diagram of a ram in the embodiment of the invention shown in Fig. 3 in contact with a bulging surface of a bladder.

Fig. 5 is a schematic close-up diagram of a portion of another embodiment for applying a squeezing pressure onto the bulging surface of a bladder.

Fig. 6 is an exploded side view of another embodiment of the cell concentration and fusion apparatus of the invention.

Fig. 7 is a rear view of the embodiment of the invention shown in Fig. 6 that is fully assembled.

Fig. 8 is a top view of a filter used in the embodiment of the invention shown in Figs. 6 and 7.

Fig. 9 is a top view of a gasket used in the embodiment of the invention shown in Figs. 6-8.

Fig. 10 is a top view of a back block used in the embodiment of the invention shown in Figs. 6-9.

Fig. 11 is an enlarged schematic diagram of a portion of a cell concentration and fusion apparatus of the invention in which electrodes are oriented parallel to the flow path of liquid past the electrodes.

Fig. 12 is an enlarged schematic diagram of a portion of a cell concentration and fusion apparatus of

the invention in which electrodes are oriented perpendicular to the flow path of liquid past the electrodes.

Modes for Carrying Out the Invention

A tangential flow, cell concentration and fusion apparatus 12 and method of use are provided. The cell concentration and fusion apparatus 12 includes a housing 24, and a first input channel 1 is contained in the housing 24. A concentration chamber 26 includes a proximal end connected to the first input channel 1 and is contained in the housing 24. The concentration chamber 26 extends along a longitudinal axis 28 through the housing 24. A first output channel 5 is contained in the housing 24 and is connected to a distal end of the concentration chamber 26. A filter 3 serves as a wall of the concentration chamber 26. The filter 3 is located between the first input channel 1 and the first output channel 5. The filter 3 extends in a direction parallel to the longitudinal axis 28. An effluent chamber 34 is contained in the housing 24. The filter 3 serves as a wall of the effluent chamber 34. A second output channel 4 is connected to the effluent chamber 34. Electrodes 6 are supported by the housing 24, the electrodes 6 are located between the first input channel 1 and the first output channel 5. The housing 24 is made of transparent material. The electrodes 6 are located adjacent to the first output channel 5, and, in one configuration, the electrodes 6 are oriented parallel to the longitudinal axis 28.

The filter 3 is a porous membrane. The filter 3 has a porosity which prevents biological cells from passing through but permits liquids to pass through.

A second input channel 2 is contained in the housing 24, and the second input channel 2 is connected to the

effluent chamber 34. The second input channel 2 serves as a vent channel.

Operation of the embodiment of the invention shown in Fig. 1 is as follows. A mixture of biological cells in a liquid carrier is introduced into the first input channel 1. The mixture passes through the first input channel 1 into the concentration chamber 26 in the direction shown by directional arrow 7. Once in the concentration chamber 26, a portion of the liquid carrier passes through the filter 3 into the effluent chamber 34. Since the biological cells are retained by the filter 3 and some of the carrier liquid is separated from the mixture, the concentration of the biological cells in the mixture increases. As the biological cells flow toward the first output channel 5, more liquid carrier is separated through the filter 3, and the biological cells become more concentrated when they reach the electrodes 6. At the electrodes 6, the concentrated cells are subjected to the application of electric fields from the voltage waveform generator 36 through wires 8. As a result of the application of the electric fields, the concentrated biological cells undergo cell fusion.

Although the concept of tangential flow past a filter is known, in the context of the subject invention, a further clarification of the meaning of tangential flow is desired. More specifically, a portion of the cell/liquid mixture in a solid/liquid separation chamber in the cell concentration and fusion apparatus is moved in a first direction towards an electrical treatment region. A portion of the liquid in the solid/liquid separation chamber is separated from the cell/liquid mixture through the filter in a second direction, which is perpendicular to the first direction. Stated somewhat differently, the filter is oriented parallel to the direction of flow of the cell/liquid mixture, and the cell/liquid mixture flows tangentially along the surface of the filter. However, the liquid which separates from the cell/liquid mixture

through the filter moves away from the cell/liquid mixture in a direction perpendicular to the direction of flow of the cell/liquid mixture.

The tangential filtration and concentration system of the invention is designed for cells to flow along the surface of the filter membrane. This apparatus and method insure that there are fewer tendencies for the filter membrane to be blocked by the biological cells. The apparatus and method also prevent excessive packing of the cells on the filter membrane. The outflow from the concentration chamber 26 is restricted or blocked at the first output channel 5 to encourage accumulation of cells in the region of the electrodes 6. Following entry of the cell/liquid mixture into the concentration chamber 26, introducing varying amounts of fluid into the first input channel 1 will increase and control cell packing density. The introduction of extra fluid will encourage further tangential flow of cells toward the electrodes 6.

The cell concentration and fusion apparatus 12 of the invention can be used in a wide variety of protocols for carrying out electrofusion and/or electroporation of biological cells. More specifically with respect to electrofusion, electrofusion is the fusion of two or more cells using pulsed electric fields. The membrane effects resulting from applied pulsed electrical fields in electrofusion are similar to those in electroporation. The principle difference is that membranes are in close contact permitting them to fuse together in the process of pore formation. For this reason, electric field densities used in electrofusion are similar to those used in electroporation.

Electrofusion is done in three major steps. First, cells are brought into contact with other cells. This is done for the devices herein using cell concentration and/or dielectrophoresis. Dielectrophoresis is a force on cells created in non-homogeneous (divergent) electric fields. The divergent electric field induces movement

toward higher field densities, in the direction of the electrodes. The movement is independent of polarity and can occur in alternating current fields.The second step in electrofusion is to apply one or more high voltage pulses to the cells, inducing membrane fusion. The voltage required must be above a threshold to induce membrane breakdown and below a maximum voltage that would cause cell death. Threshold voltage is approximately one volt across the cell membrane or two volts across two cell's membranes. The voltage across a cell is equal to 1.5 times the cell radius times the electric field strength times the cosine of the angle of the membrane in relation of the direction of the field. This is the same formula used for electroporation. Multiple fusion pulses may be more efficient than a single pulse.The last step in the electrofusion process (when using dielectrophoresis as an alignment tool) is post fusion alignment. Electrofusion is a process that continues to occur over some time after the fusion pulse is applied. Re-applying dielectrophoresis after the fusion pulse allows maturation of the fusion process by holding cells in optimal alignment and contact.

The cell concentration and fusion apparatus 12 can be used in a number of different modes. The cell/liquid mixture can flow continuously into the cell concentration and fusion apparatus 12, and electrial-field-treated cells can flow continuously out from the cell concentration and fusion apparatus 12. In addition, the cell/liquid mixture can be introduced into the cell concentration and fusion apparatus 12 by a series of batches, and the electrial-field-treated cells can flow out from the cell concentration and fusion apparatus 12 in a batchwise manner.

The following steps comprise a protocol for electrofusion which involves dielectrophoresis and intermittent flow through the cell concentration and

fusion apparatus 12, wherein fused hybridoma cells are produced.

1. Immunize mice.

2. Two weeks after the last dose of immunogen, harvest spleen cells (containing B cells).

3. Obtain cultured myeloma cells such as SP2-0 cells.

4. Count the B cells and myeloma cells. Mix together in a pre-determined ratio (1:1 to 1:5).

5. Prepare an isoosmolar, low conductance fusion medium, as follows.

a. Sugar alcohol 280 mOsM use either Sorbitol (MW 182.2) at 51 grams/L or Inositol (MW 180.2) at 50 grams/L.

b. Calcium Acetate 0.1 mM.

c. Magnesium Acetate 0.5 mM.

d. Histidine 1 mM (for pH control)

e. PH should be in the range of 7.0-7.4.

(Note: Do not adjust the pH with inorganic acids or bases as that will increase the ion concentration).

f. Filter in a sterile manner.

6. Wash the cells 3 times in the new medium. A thorough wash is necessary. Washing is accomplished buy centrifuging the cells at 400g for 10 minutes followed by pouring off the supernatant, re-suspending the pellet with a gentle flick and adding the fusion medium.

7. An alternative to the wash is medium replacement using a modified tangential flow apparatus. Two filters would be used. One for introducing new medium and another for collecting filtrate.

8. Count the cells before the last centrifugation. Re-suspend the cells at 3,000,000 cells/ml after the last centrifugation.

9. Add 100 microL cell suspension to the apparatus.

10. Add one ml cell fusion medium to the apparatus keeping the exit valve closed (e. g. third valve 45 in

Fig. 2). This step is to flush the cells to the electrode end of the apparatus.

11. Turn on the cell fusion electronic equipment (the voltage waveform generator 36). The commercial equipment made by Cyto Pulse Sciences, Inc., Columbia, MD, called a PA4000 electroporator with a PA option is used in this example.

12. Program the PA4000 for a pre-fusion sine wave dielectrophoresis alternating current of 20 volts (beginning and ending voltage) and a frequency of 2 MHz for 10 seconds.

13. Program the PA4000 for 1-2 fusion pulses at 2 kV/cm and microseconds duration each.

14. Program the PA4000 for a post fusion sine wave dielectrophoresis alternating current of 20 volts beginning to 0 volts ending (ramp down) at a frequency of 2 MHz for 10 seconds.

15. Connect the tangential flow fusion apparatus to the outlet of the PA4000 unit and initiate the protocol.

16. After the electrofusion is done, wait 10 minutes and open the exit valve and flow medium through the apparatus to collect the cells.

17. Flush the cells from the fusion electrode and place the cells in growth medium.

18. After the cells have had time to recover, in 24 to 48 hours, change the medium to a selective medium.

The following steps presented below comprise a protocol for electrofusion which involves using intermittent flow through the cell concentration and fusion apparatus 12, without using dielectrophoresis, wherein fused hybridoma cells are produced.

1. Immunize mice.

2. Two weeks after the last dose of immunogen, harvest spleen cells (containing B cells).

3. Obtain cultured myeloma cells such as SP2-0 cells.

4. Count the B cells and myeloma cells. Mix together in a pre-determined ratio (1:1 to 1:5).

5. Prepare an isoosmolar, low conductance fusion medium.

a. Sugar alcohol 280 mOsM use either Sorbitol (MW 182.2) at 51 grams/L or Inositol (MW 180.2) at 50 grams/L.

b. Calcium Acetate 0.1 mM.

c. Magnesium Acetate 0.5 mM.

d. Histidine 1 mM (for pH control).

e. PH should be in the range of 7.0-7.4. Note Do not adjust the pH with inorganic acids or bases as that will increase the ion concentration.

f. Filter in a sterile manner.

6. Wash the cells 3 times in the new medium. A thorough wash is necessary. Washing is accomplished buy centrifuging the cells at 400g for 10 minutes followed by pouring off the supernatant, re-suspending the pellet with a gentle flick and adding the fusion medium.

7. An alternative to the wash is medium replacement using a modified tangential flow apparatus of the invention. Two filters would be used. One for introducing new medium and another for collecting filtrate.

8. Count the cells before the last centrifugation. Re-suspend the cells at 1,000,000 cells/ml after the last centrifugation.

9. Add 200 microL cell suspension to the apparatus.

10. Add one ml cell fusion medium to the apparatus keeping the exit valve closed. This step is to flush the cells to the electrode end of the apapratus.

11. Turn on the cell fusion electronic equipment. The commercial apparatus made by Cyto Pulse Sciences, Inc., Columbia, MD, called a PA4000 electroporator with a PA option is used in this example.

12. Program the PA4000 for 1-2 fusion pulses at 2 kV/cm and microseconds duration each.

13. Connect the tangential flow fusion apparatus of the invention to the outlet of the PA and initiate the protocol.

14. After the electrofusion is done, wait 10 minutes and open the exit valve and flow medium through the apparatus to collect the cells.

15. Flush the cells from the fusion electrode and place the cells in growth medium.

16. After the cells have had time to recover, in 24 to 48 hours, change the medium to a selective medium.

Turning to Fig. 2, a second embodiment of the invention is shown. Reference numerals are shown that correspond to like reference numerals that designate like elements shown in the other figures. Cell mixture bladder 40 is connected to the first input channel 1. A liquid bladder 42 is connected to the first input channel 1. A fused cell bladder 44 is connected to the first output channel 5, and an exhaust bladder 46 is connected to the second output channel 4. A first valve 41 is located between the cell mixture bladder 40 and the first input channel 1. A second valve 43 is located between the liquid bladder 42 and the first input channel 1, and a third valve 45 is located between the fused cell bladder 44 and the first output channel 5. The first valve 41, second valve 43, and the third valve 45 can be embodied in different forms. For example, they can be simply in the form of flexible tubes that are pinched appropriately by an electromechanical clamp controlled by the bladder assemblies controller 49. Alternatively, the valves can be automatically operating one-way check valves.

An input port 9 is connected to the cell mixture bladder 40. An injection output port 10 is connected to the fused cell bladder 44, and an electrical connection jack 14 is electrically connected to the wires 8. The input port 9 and the injection output port 10 are made from self-sealing elastomeric material. In this respect,

the input port 9 is an injection input port 9, and the output port 10 is an injection output port 10. The input port 9 and the injection output port 10 are suitable for receiving a hypodermic needle.

Alternatively, the input port 9 and the output port 10 can be equipped with valves so that a continuous input of cell/liquid mixture can be introduced through the input port 9 and so that a continuous output of fused biological cells can be produced from the output port 10. Also, with valves at the input port 9 and the output port 10, cell/liquid mixtures can be processed through the cell fusion module 20 in a series of batches.

A bag 13 envelopes the cell concentration and fusion apparatus 12, the cell mixture bladder 40, the liquid bladder 42, the fused cell bladder 44, the exhaust bladder 46, and the wires 8 for providing a cell fusion module 20. The bag 13 leaves access to the input port 9, the injection output port 10, and the electrical connection jack 14. The bag 13 is hermetically sealed. The interior of the cell fusion module 20 is maintained in a sterile status by the bag 13. The bag 13 is made from flexible material.

Turning to Fig. 3, a third embodiment of the invention is shown. Reference numerals are shown that correspond to like reference numerals that designate like elements shown in the other figures. In addition, a module manipulator unit 22 is provided for receiving and operating the cell fusion module 20. The module manipulator unit 22 includes a manipulator housing 48. A first bladder operation assembly 50 is supported by the manipulator housing 48 for operating the cell mixture bladder 40. A second bladder operation assembly 52 is supported by the manipulator housing 48 for operating the liquid bladder 42, and a bladder assemblies controller 49 is provided for controlling the first bladder operation assembly 50 and the second bladder operation assembly 52. The bladder assemblies controller 49 can be programmable.

A third bladder operation assembly 54 is supported by the manipulator housing 48 for operating the fused cell bladder 44, and a fourth bladder operation assembly 56, supported by the manipulator housing 48, is provided for operating the exhaust bladder 46. The third bladder operation assembly 54 and the fourth bladder operation assembly 56 are controlled by the bladder assemblies controller 49. It is noted that the third bladder operation assembly 54 and the fourth bladder operation assembly 56 may be considered optional. With certain operating conditions of the cell fusion module 20, the fused cell bladder 44 and the exhaust bladder 46 may be filled simply as a result of pressure applied to the cell mixture bladder 40 by the first bladder operation assembly 50 and/or pressure applied to the liquid bladder 42 by the second bladder operation assembly 52.

On the other hand, under different operating conditions of the cell fusion module 20, it may be desirable to force liquid from the exhaust bladder 46 back through the filter 3 to backflush the filter 3 and dislodge biological cells from the filter 3. Under those conditions, the fourth bladder operation assembly 56 would be employed.

Similarly, there may be conditions under which it would be desirable to force concentrated cells out from the fused cell bladder 44. Under these conditions, the third bladder operation assembly 54 would be employed to do just that.

Each of the bladder operation assemblies includes a respective shaft drive unit 60 driven by the bladder assemblies controller 49. A respective shaft 62 is driven by the shaft drive unit 60, and a respective ram 64 is moved by the shaft 62. The third embodiment of the invention, shown in Fig. 3, is suitable for clinical applications. Briefly, the self-contained cell fusion module 20 is placed in the module manipulator unit 22, and the voltage waveform generator 36 is connected to the

electrical connection jack 14. A mixture containing biological cells and a liquid are injected through the injection input port 9 into the cell mixture bladder 40 using a hypodermic needle and syringe.

Detailed operation of the module manipulator unit 22 is controlled by the bladder assemblies controller 49. More specifically, the bladder assemblies controller 49 first controls the first bladder operation assembly 50 to push a portion of the cell/liquid out of the cell mixture bladder 40, through the first valve 41 and into the cell concentration and fusion apparatus 12. To do so, the shaft drive unit 60 of the first bladder operation assembly 50 pushes the associated shaft 62 to push the associated ram 64 against the flexible bag 13 which pushes against the cell mixture bladder 40. A portion of the cell/liquid mixture is pushed through the first input channel 1 and into the concentration chamber 26. Some of the liquid passes through the filter 3, whereby the biological cells in the cell/liquid mixture are concentrated, and whereby the filtered liquid passes into the effluent chamber 34. The concentrated cells are pulsed at the electrodes 6 by the voltage waveform generator 36 which is electrically connected to the electrical connection jack 14.

After the concentrated cells have been pulsed, a portion of the concentrated cells are fused together. The bladder assemblies controller 49 then operates the second bladder operation assembly 52. The liquid bladder 42 contains a phosphate-buffered saline solution (PBS), and the second bladder operation assembly 52 forces a quantity of the PBS through the second valve 43 and into the concentration chamber 26 so that the fused cells are forced through the third valve 45 and into fused cell bladder 44. A quantity of fused cells can be removed from the fused cell bladder 44 by inserting a hypodermic needle into the injection output port 10.

In Fig. 4, the close-up diagram shows a ram 64 in the embodiment of the invention shown if Fig. 3 in contact with a bulging surface 65 of a bladder. The bladder is squeezed by mechanical compresion when the ram 64 compresses the bulging bladder surface 65.

In Fig. 5, the close-up diagram shows a portion of another embodiment for applying a squeezing pressure onto the bulging surface 65 of a bladder. More specifically, driving bladder 67 is placed over the bulging surface 65 of an underlying bladder. The driving bladder 67 contains a hydraulic fluid, such as water 69. Hydraulic pressure is applied to the driving bladder 67 through and intake tube 71. With this embodiment, it is understood that the respective bladder operation assembly includes means for applying hydraulic pressure, such as a pump or a piston/cylinder combination.

Turning to another electrofusion protocol, the following steps comprise a protocol for electrofusion which involves a clinical application using the cell fusion module 20 and the module manipulator unit 22 of the invention shown in Figs. 2 and 3. More specifically, this protocol carries out the fusing of human dendritic cells and tumor cells.

1. Obtain and culture dendritic cells using published methods (Schuler et al and Romani et al).

Schuler, G, Lutz, M, Bender, A, Thurner, B, Roder, C, Young, JW, Romani, N. A guide to the Isolation and propagation of dendritic cells, pages 515- 533, in Dendritic Cells, editors Lotzem MT, Thompson, AW, Academic Press, 19.

Romani, N, Reider, D, Heuer, M, Eibl, B, Niederwieser, D, Schuler, G. (1996). Generation of mature dendritic cells from human blood an improved method with special regard to clinical applicability. J. Immunol. Methods 196, 137-151.

2. Obtain and culture human tumor cells. The isolation and culture technique will be tissue type dependent and several standard techniques are available.

3. Remove a sample of dendritic cells for the fusion.

4. Remove a sample of human tumor cells.

5. Irradiate the tumor cells to render them incapable of cell division. The amount of irradiation will be cell type specific.

6. Prepare an isoosmolar, low conductance fusion medium which includes:

a. Sugar alcohol 280 mOsM use either Sorbitol (MW 182.2) at 51 grams/L or Inositol (MW 180.2) at 50 grams/L.

b. Calcium Acetate 0.1 mM.

c. Magnesium Acetate 0.5 mM.

d. Histidine 1 mM (for pH control)

e. PH should be in the range of 7.0-7.4.

(Note: Do not adjust the PH with inorganic acids or bases as that will increase the ion concentration.

f. Filter in a sterile manner.

7. Prepare complete medium which includes:

a. Dulbeco's Modified Essential Medium (DMEM).

b. 10% heat inactivated human serum AB male.

c. L-glutamine X supplement, 5ml/500 ml.

8. Wash each of the cell types (dendritic cells and tumor cells) 3 times in the fusion medium. A thorough wash is necessary. Washing is accomplished by centrifuging the cells at 400g for 10 minutes followed by pouring off the supernatant, re-suspending the pellet with a gentle flick and adding the fusion medium.

9. Count the cells before the last centrifugation.

10. Mix the cells in a pre-determined ratio.

11. Add fusion medium and centrifuge cells at 400 g for 10 minutes.

12. Re-suspend the cells at 300,000 cells/ml after the last centrifugation.

13. Inject one ml of cell suspension to the intake port 9.

14. Place the clinical fusion device into the device holder.

15. Initiate the fusion protocol.

16. Remove the clinical fusion device (that is the cell fusion module 20) when the indicator shows that the fusion protocol is complete.

17. Insert a syringe needle into the output port 10 and remove the fused cells.

18. Add the cells to a 15 ml conical tube with 3 ml complete medium.

19. Wait 15 minutes then centrifuge the cells at 400 g for 10 minutes.

20. Pour off the supernatant and add 7 ml complete medium.

21. Add cell suspension to a 25 ml tissue culture flask and culture until needed.

In Fig. 6, an exploded side view of another embodiment of the cell concentration and fusion apparatus of the invention is shown. Figs. 7, 8, 9 and 10 show different views of this embodiment. The cell concentration and fusion apparatus 12 includes a base block 21, which is made from clear polycarbonate and that has the following dimensions, 70 mm. X 40 mm. X 10 mm.. A front block 23 is made from clear polycarbonate, has the following dimensions, 60 mm. X 40 mm. X 10 mm., and is supported by the base block 21. The front block 23 includes a milled front slot 25 which has the following dimensions, 40 mm. X 3 mm. X 3 mm., includes a vent channel 27 which is a drilled hole 0.8 mm. in diameter and 10 mm. deep, includes front fastener-reception channels 55, and includes a drain channel 29 which is a through hole 1 mm. in diameter.

A back block 31 is made from clear polycarbonate, has the following dimensions, 60 mm. X 40 mm. X 20 mm.,

and is supported by the base block 21. The back block 31 includes an input channel 33 which is a 0.8 mm. diameter hole 11 mm. deep and includes a transverse hole 35 in communication with the input channel 33. The transverse hole 35 is 1 mm. in diameter and 1.8 mm. deep. The back block 31 includes a milled back slot 37 in communication with the transverse hole 35. The back slot 37 has the following dimensions, 40 mm. X 2 mm. X 1 mm.. The back block 31 includes an electrode 39 which is a 1 mm. X 1 mm. platinum bar, includes an output channel 51 which is a hole 0.8 mm. in diameter and 21 mm. deep from the side and is in communication with the back slot 37. The back block 31 also includes a pair of electrical jacks 53 connected to the electrode 39 and includes back fastener-reception channels 57.

A gasket 72 located adjacent to the front block 23 between the front block 23 and the back block 31. The gasket 72 is made from transparent silicone rubber, and has the following dimensions 0.5 mm. X 60 mm. X 40 mm.. The gasket 72 includes gasket fastener-reception channels 74, and includes a filter access channel 76 which has dimensions 2 mm. X 40 mm..

A filter 63 has dimensions 50 mm. X 10 mm. X 0.0008 mm. and is located between the gasket 65 and the back block 31. Fasteners 61 are received in the back fastener-reception channels 57, the gasket fastener-reception channels 74, and the front fastener-reception channels 55 when the back fastener-reception channels 57, the gasket fastener-reception channels 74, and the front fastener-reception channels 55 are placed in registration.
In Fig. 11, an enlarged schematic diagram of a portion of a cell concentration and fusion apparatus of the invention is shown in which electrodes are oriented parallel to the flow path of liquid past the electrodes. This embodiment of the invention is shown in detail in Fig. 1.

Fig. 12, an enlarged schematic diagram of a portion of a cell concentration and fusion apparatus of the

invention is shown in which electrodes are oriented perpendicular to the flow path 7 of liquid past the electrodes. That is, the electrodes 15 are supported by the housing 24 and are oriented perpendicular to the longitudinal axis 28. In Fig. 12, the electrodes 15 are shown as screen electrodes 15.

Although the apparatuses of the invention have been discussed primarily in the context of cell fusion, it is also contemplated that biological cells can be concentrated with the invention prior to subjecting the cells to electroporation for the uptake or delivery of DNA, RNA, peptide, or protein material so that smaller quantities of the DNA, RNA, peptide, or protein material can be employed.

From the above description, it is evident that a novel method for the concentration and electrical treatment of biological cells is provided by the invention. Generally, the method of concentrating and electrically treating biological cells includes the steps of:

obtaining a mixture of the biological cells in a liquid carrier,

moving a portion of the cell/liquid mixture into a solid/liquid separation chamber in a first direction towards an electrical treatment region,

separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells, and

collecting the biological cells that have been concentrated and treated with the electrical field.

The method of the invention includes the step of applying an electrical field to the concentrated biological cells. In this respect, the method of the invention can include applying an electrical field to bring about dielectrophoresis in the concentrated biological cells, can include applying an electrical field to bring about cell fusion in the concentrated biological cells, and can include applying an electrical field to bring about dielectrophoresis in the fused cells to result in post-fusion maturation of the fused cells.

The electrical field that is applied to the concentrated cells can bring about cell fusion. Alternatively, the electrical field that is applied to the concentrated cells and bring about electroporation.

In the context of employing the cell fusion module 20 and the module manipulator unit 22, a method of concentrating and electrically treating biological cells includes the steps of:

obtaining a cell fusion module which includes a cell concentration and fusion apparatus,

placing the cell fusion module in a module manipulator unit,

obtaining a mixture of the biological cells in a liquid carrier,

introducing a portion of the cell/liquid mixture into the cell concentration and fusion apparatus 12, under control of the module manipulator unit 22,

moving a portion of the cell/liquid mixture into a solid/liquid separation chamber in the cell concentration and fusion apparatus 12, in a first direction, towards an electrical treatment region in the cell concentration and fusion apparatus 12,

separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first

direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells inside the cell concentration and fusion apparatus 12,

moving the concentrated electrial-field-treated cells from the cell concentration and fusion apparatus to a collection region inside the cell fusion module 20,

removing the electrial-field-treated cells from the collection region inside the cell fusion module 20 to a location outside the cell fusion module 20.

Additionally, the cell fusion module 20 can be considered disposable and can be disposed of in its entirety after the fused cells have been collected.

It is apparent from the above that the present invention accomplishes all of the objects set forth by providing a new and improved tangential flow, cell concentration and fusion apparatus that is relatively simple in design and operation, and which advantageously employs a concentrating porous filter which is not oriented perpendicular to the direction of liquid flow through the apparatus. With the invention, a tangential flow, cell concentration and fusion apparatus provides both unobstructed liquid flow and concentration of biological cells that are to undergo electrofusion. With the invention, a tangential flow, cell concentration and fusion apparatus provides an electroporation or electrofusion apparatus which employs tangential flow filtration. With the invention, a tangential flow, cell concentration and fusion apparatus is provided which can be contained in a sealed, sterile system. With the invention, a tangential flow, cell concentration and fusion apparatus is provided which can be contained in a module which can be used, disposed of, and replaced in a simple and easy manner. With the invention, a tangential

flow, cell concentration and fusion apparatus is provided which can employ a module manipulator that interacts with and operates a cell fusion module from outside the module without entering the module so that the sterility inside the module is not disturbed.

Claims

What is claimed is:

1. A cell concentration and fusion apparatus (12), comprising:

a housing (24),

a first input channel (1) contained in said housing (24),

a concentration chamber (26) which includes a proximal end connected to said first input channel (1) and contained in said housing (24), wherein said concentration chamber (26) extends along a longitudinal axis (28) through said housing (24),

a first output channel (5) contained in said housing (24) and connected to a distal end of said concentration chamber (26),

a filter (3) which serves as a wall of said concentration chamber (26), wherein said filter (3) is located between said first input channel (1) and said first output channel (5), wherein said filter (3) extends in a direction parallel to said longitudinal axis (28),

an effluent chamber (34) contained in said housing (24), wherein said filter (3) serves a wall of said effluent chamber (34),

a second output channel (4) connected to said effluent chamber (34),

electrodes (6) supported by said housing (24), wherein said electrodes (6) are located between said first input channel (1) and said first output channel (5).

2. The apparatus of claim 1 wherein said housing (24) is made of transparent material.

3. The apparatus of claim 1 wherein said electrodes (6) are located adjacent to said first output channel (5).

4. The apparatus of claim 1 wherein said electrodes (6) are oriented parallel to said longitudinal axis (28).

5. The apparatus of claim 1 wherein said filter (3) is a porous membrane.

6. The apparatus of claim 1 wherein said filter (3) has a porosity which prevents biological cells from passing through but permits liquids to pass through.

7. The apparatus of claim 1 , further including:

a second input channel (2) contained in said housing (24), wherein said second input channel (2) is connected to said effluent chamber (34).

8. The apparatus of claim 1 wherein said second input channel (2) serves as a vent channel.

9. The apparatus of claim 1 , further including:

a cell mixture bladder (40) connected to said first input channel (1),

a liquid bladder (42) connected to said first input channel (1),

a fused cell bladder (44) connected to said first output channel (5), and

an exhaust bladder (46) connected to said second output channel (4).

10. The apparatus of claim 9, further including:

a first valve (41) between said cell mixture bladder (40) and said first input channel (1),

a second valve (43) between said liquid bladder (42) and said first input channel (1), and

a third valve (45) between said fused cell bladder (44) and said first output channel (5).

11. The apparatus of claim 9 , further including:

an injection input port (9) connected to said cell mixture bladder (40),

an injection output port (10) connected to said fused cell bladder (44), and

an electrical connection jack (14) electrically connected to said wires (8).

12. The apparatus of claim 11 wherein said injection input port (9) and said injection output port (10) are made from self-sealing elastomeric material.

13. The apparatus of claim 11, further including:

a bag (13) which envelopes said cell concentration and fusion apparatus (12), said cell mixture bladder (40), said liquid bladder (42), said fused cell bladder (44), said exhaust bladder (46), and said wires (8) for providing a cell fusion module (20), wherein said bag (13) leaves access to said injection input port (9), said injection output port (10), and said electrical connection jack (14).

14. The apparatus of claim 13 wherein said bag (13) is hermetically sealed.

15. The apparatus of claim 13 wherein the interior of said cell fusion module (20) is maintained in a sterile status by said bag (13).

16. The apparatus of claim 13 wherein said bag (13) is made from flexible material.

17. The apparatus of claim 13, further including:

a module manipulator unit (22) for receiving and operating said cell fusion module (20).

18. The apparatus of claim 17 wherein said module manipulator unit (22) includes:

a manipulator housing (48),

a first bladder operation assembly (50) supported by said manipulator housing (48) for operating said cell mixture bladder (40),

a second bladder operation assembly (52) supported by said manipulator housing (48) for operating said liquid bladder (42), and

a bladder assemblies controller (49) for controlling said first bladder operation assembly (50), and said second bladder operation assembly (52).

19. The apparatus of claim 18, further including:

a third bladder operation assembly (54) supported by said manipulator housing (48) for operating said fused cell bladder (44), and

a fourth bladder operation assembly (56) supported by said manipulator housing (48) for operating said exhaust bladder (46),

wherein said third bladder operation assembly (54) and said fourth bladder operation assembly (56) are controlled by said bladder assemblies controller (49).

20. The apparatus of claim 19 wherein each of said first bladder operation assembly (50), said second bladder operation assembly (52), said third bladder operation assembly (54), and said fourth bladder operation assembly (56) includes a respective shaft drive unit (60) driven by said bladder assemblies controller (49), a respective shaft (64) driven by said shaft drive unit (60), and a respective ram (64) moved by said shaft (64).

21. A cell concentration and fusion apparatus (12), comprising:

a base block (21),

a front block (23) that is supported by said base block (21), wherein said front block (23) includes a

milled front slot (25), includes a vent channel (27), includes front fastener-reception channels (55) and includes a drain channel (29),

a back block (31) that is supported by said base block (21), wherein said back block (31) includes an input channel (33), includes a transverse hole (35) in communication with said input channel (33), includes a milled back slot (37) in communication with said transverse hole (35), includes an electrode (39), includes an output channel (51) in communication with said back slot (37), includes a pair of electrical jacks (53) electrically connected to said electrode (39), and includes back fastener-reception channels (57),

a gasket (72) located adjacent to said front block (23) between said front block (23) and said back block (31), wherein said gasket (72) includes gasket fastener-reception channels (74), and includes a filter access channel (76)

a filter 63 located between said gasket (65) and said back block (31),

fasteners (61) received in said back fastener-reception channels (57), said gasket fastener-reception channels (74), and said front fastener-reception channels (55) when said back fastener-reception channels (57), said gasket fastener-reception channels (74), and said front fastener-reception channels (55) are placed in registration.

22. The apparatus of claim 21 wherein said electrodes (15) are supported by said housing (24) and are oriented perpendicular to said longitudinal axis (28).

23. A method of concentrating and electrically treating biological cells, comprising the steps of:

obtaining a mixture of the biological cells in a liquid carrier,

moving a portion of the cell/liquid mixture in a solid/liquid separation chamber in a first direction towards an electrical treatment region,

separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells,

collecting the biological cells that have been concentrated and treated with the electrical field.

24. The method of claim 23 wherein said step of applying an electrical field to the concentrated biological cells includes the steps of:

applying an electrical field to bring about dielectrophoresis in the concentrated biological cells,

applying an electrical field to bring about cell fusion in the concentrated biological cells, and

applying an electrical field to bring about dielectrophoresis in the fused cells to result in post-fusion maturation of the fused cells.

25. The method of claim 23 wherein the electrical field that is applied to the concentrated cells brings about cell fusion.

26. The method of claim 23 wherein the electrical field that is applied to the concentrated cells brings about electroporation.

27. A method of concentrating and electrically treating biological cells, comprising the steps of:

obtaining a cell fusion module which includes a cell concentration and fusion apparatus,

placing the cell fusion module in a module manipulator unit (22),

obtaining a mixture of the biological cells in a liquid carrier,

introducing a portion of the cell/liquid mixture into the cell concentration and fusion apparatus (12),

under control of the module manipulator unit (22), moving a portion of the cell/liquid mixture in a solid/liquid separation chamber in the cell concentration and fusion apparatus (12) in a first direction towards an electrical treatment region,

in the cell concentration and fusion apparatus (12), separating a portion of the liquid in the solid/liquid separation chamber from the cell/liquid mixture in a second direction, which is perpendicular to the first direction, as the cell/liquid mixture moves toward the electrical treatment region in the first direction, whereby the biological cells are concentrated in the liquid remaining in the solid/liquid separation chamber,

applying an electrical field to the concentrated biological cells inside the cell concentration and fusion apparatus (12), and

moving the concentrated electrial-field-treated cells out from the cell concentration and fusion apparatus.

28. The method of claim 27 wherein the cell/liquid mixture is moved through the cell fusion module (20) in a continuous manner.

29. The method of claim 27 wherein the cell/liquid mixture is moved through the cell fusion module (20) in a batchwise manner.

30. The method of claim 27 wherein the electrial-field-treated cells are moved to a collection region inside the cell fusion module (20).

31. The methdod of claim 30, further including the step of removing the electrial-field-treated cells from the collection region inside the cell fusion module (20) to a location outside the cell fusion module (20).

32. The method of claim 31, further including the step of disposing of the cell fusion module (20).

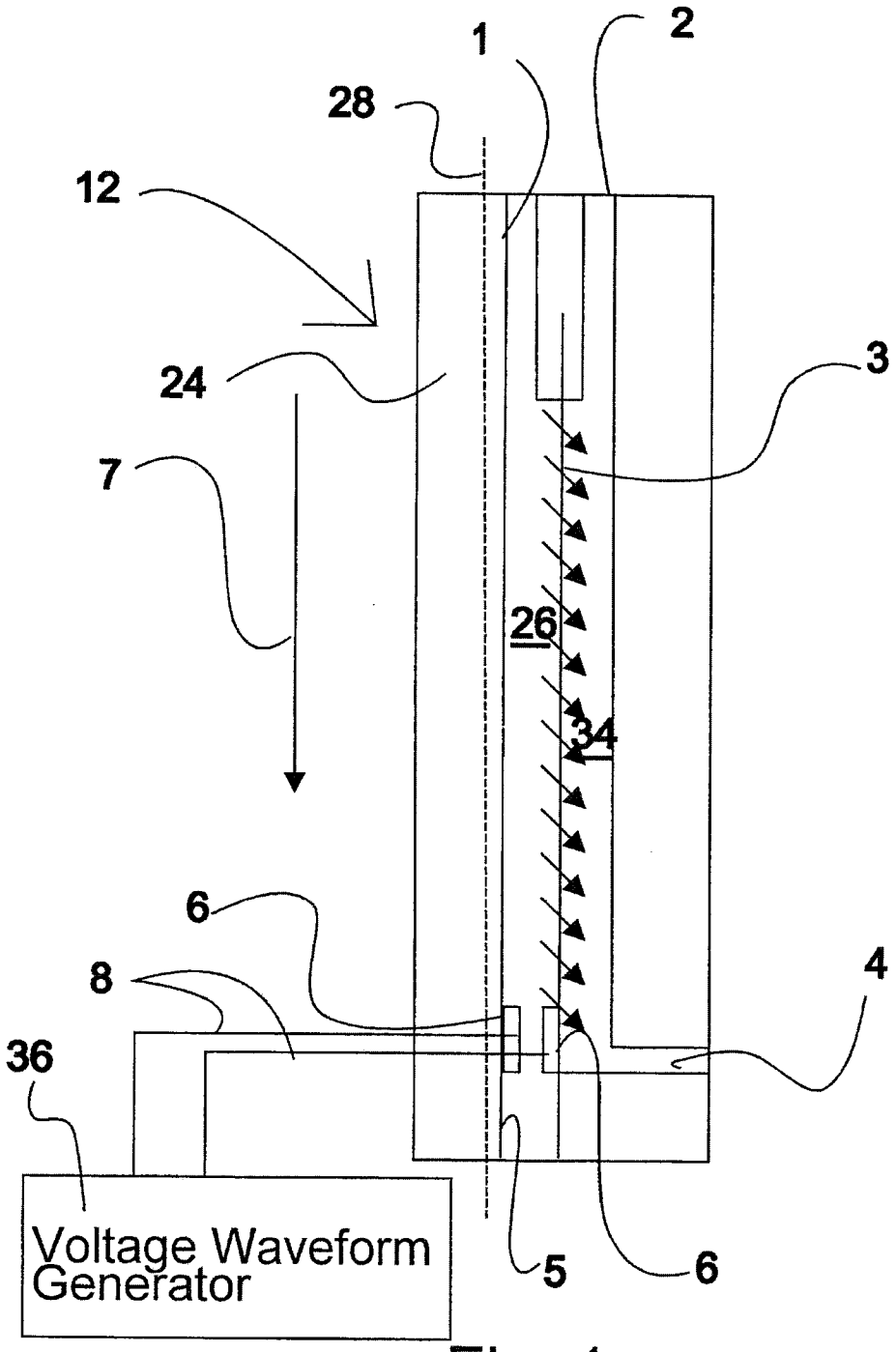

Fig. 1

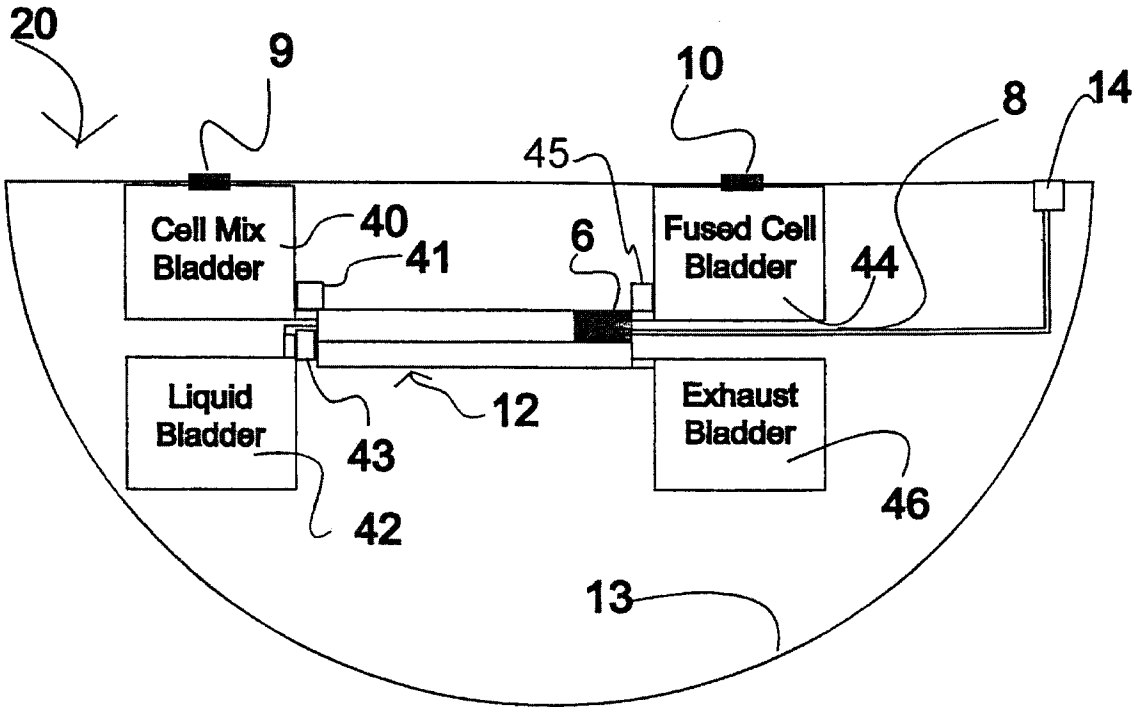
20
9
10
8
14
45
Cell Mix Bladder
40
41
6
Fused Cell Bladder
44
Liquid Bladder
43
12
Exhaust Bladder
42
46
13

Fig. 2

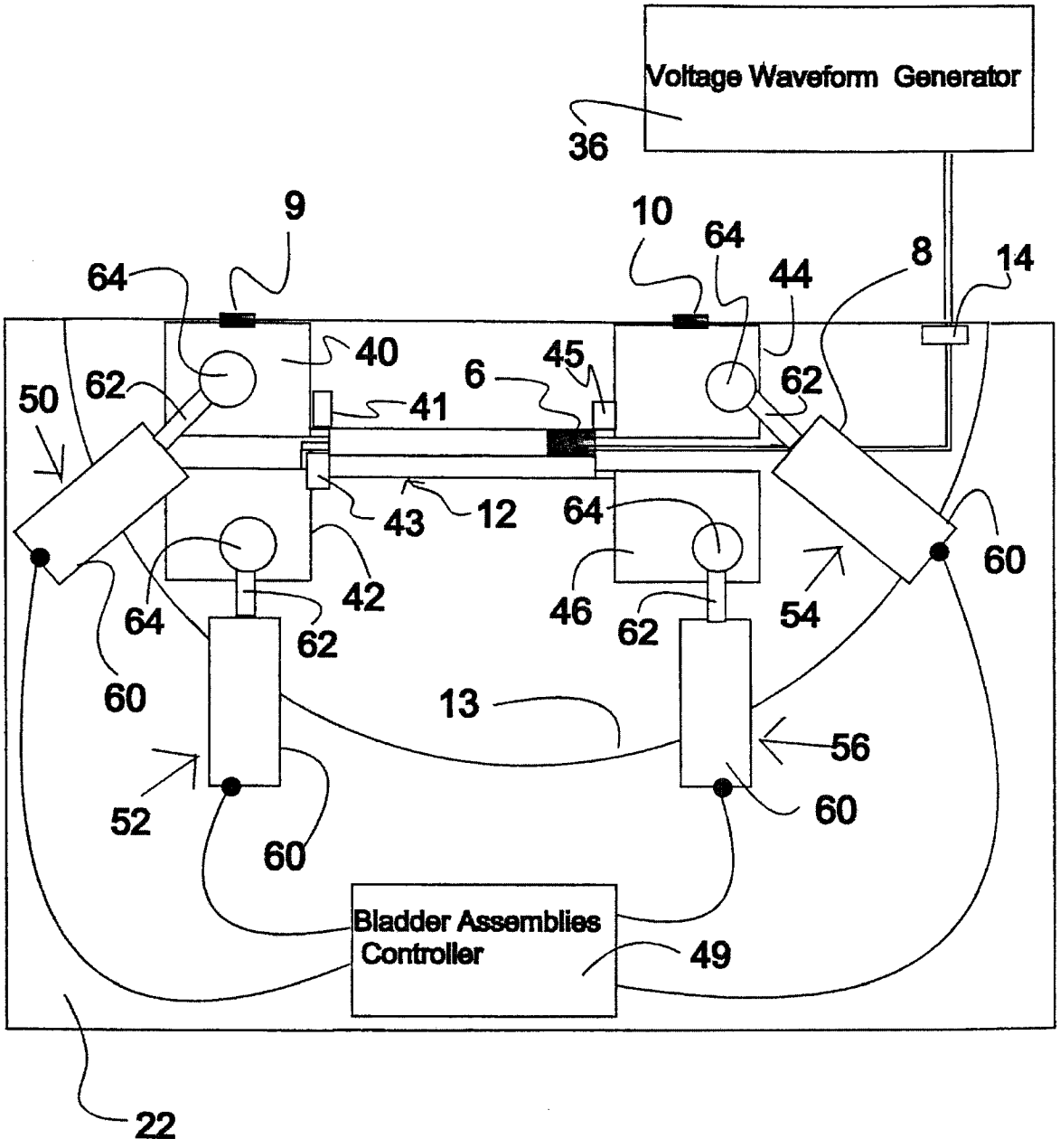
Voltage Waveform Generator
36
9
10
64
64
44
8
14
40
45
6
62
62
50
41
43
12
64
42
64
62
46
62
54
60
64
60
13
56
52
60
60
Bladder Assemblies Controller
49
22

Fig. 3

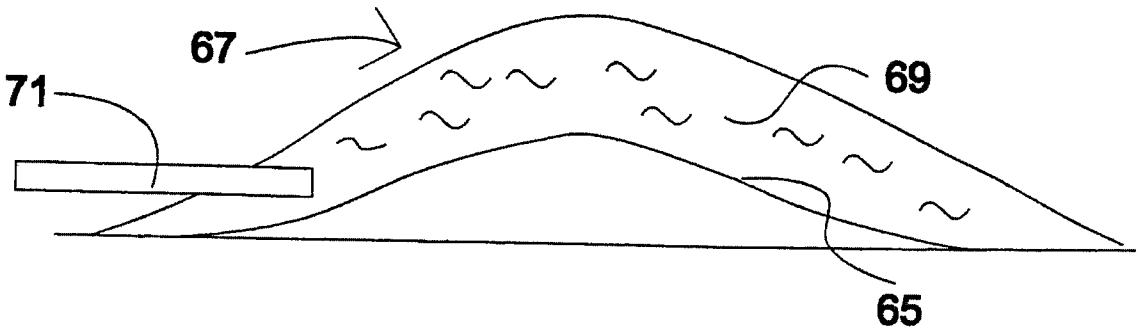

Fig. 5

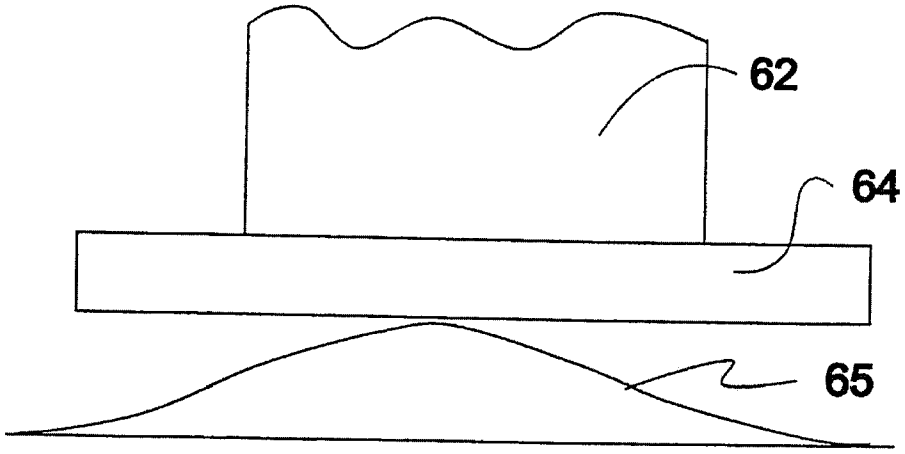

Fig. 4

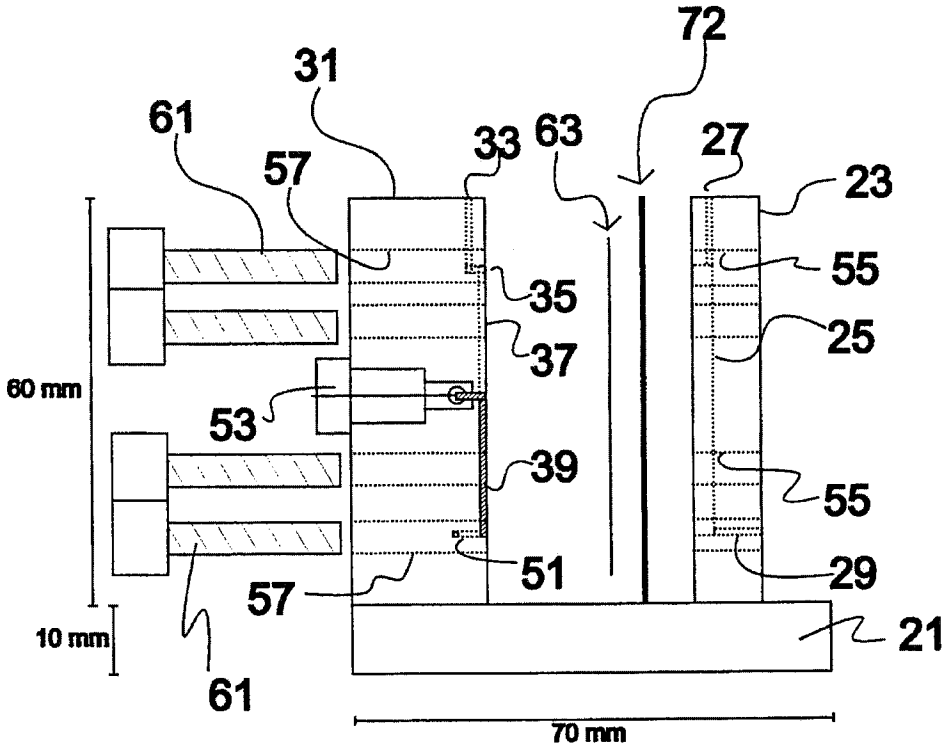
72
31
61
33
63
27
23
57
55
35
25
37
60 mm
53
39
55
51
29
57
10 mm
21
61
70 mm

Fig. 6

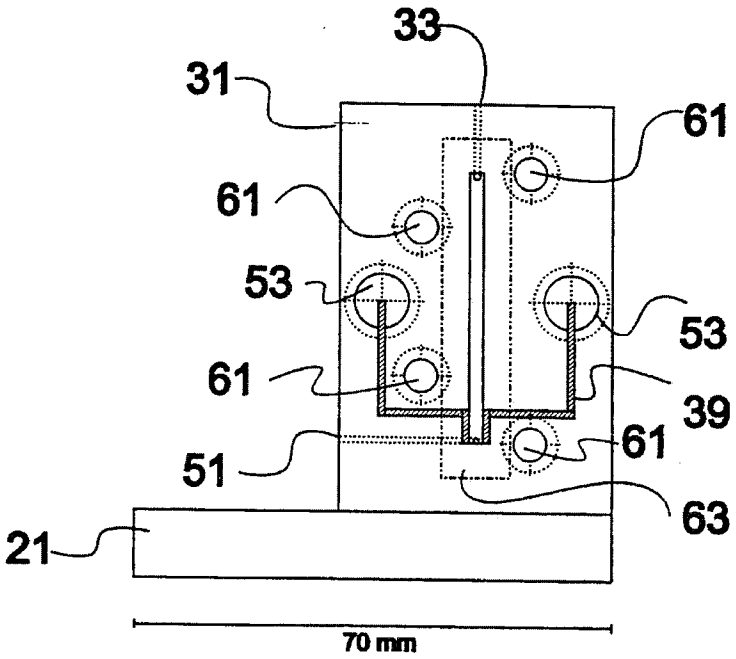

Fig. 7

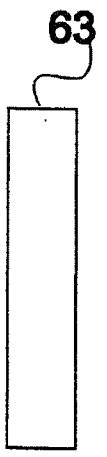

Fig. 8

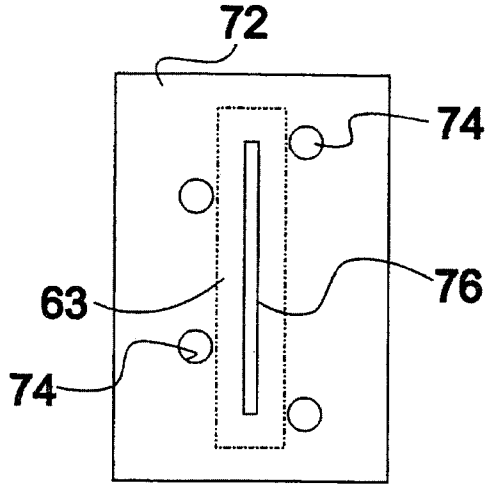

Fig. 9

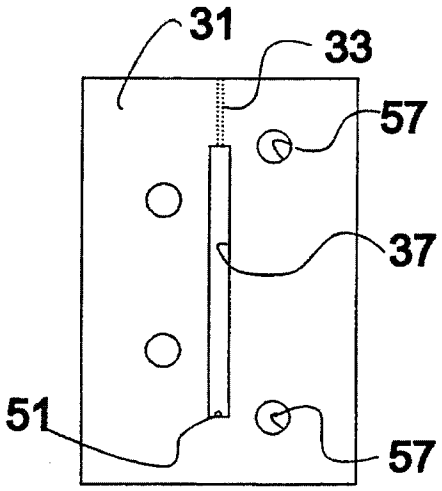

Fig. 10

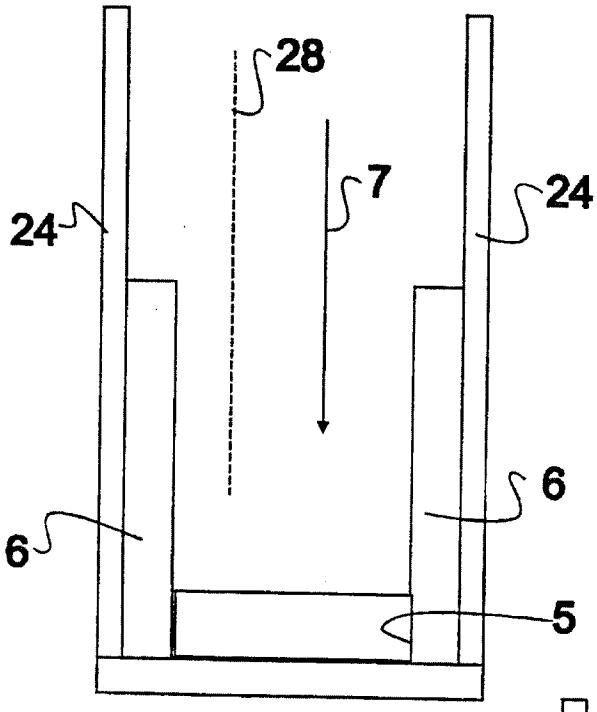

Fig. 11

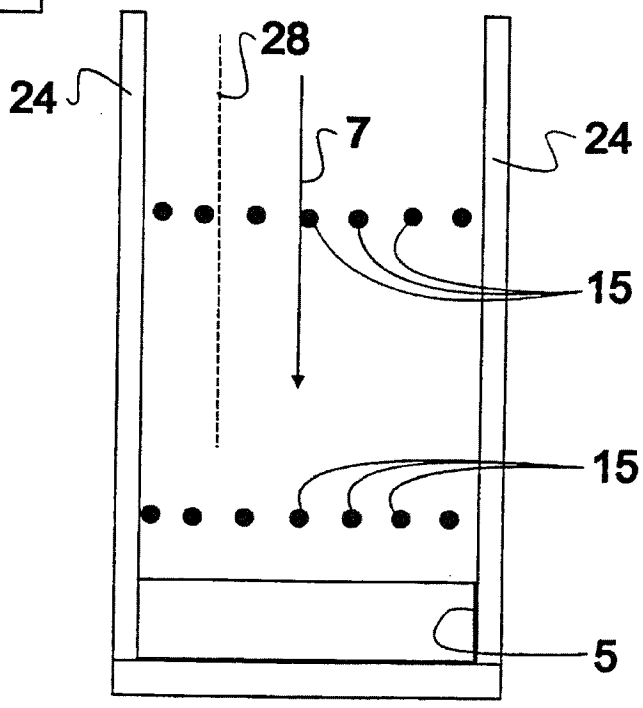

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/06420

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 13/00, C12M 1/00; B01D 57/02; C02F 1/40
US CL :435/173.7, 285.2; 204/547, 643

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/173.7, 285.2; 204/547, 643

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,832,814 A (ROOT) 23 May 1989, see description of figure 1.	1-32
A,P	US 6,010,613 A (WALTERS et al.) 04 January 2000	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search
07 JUNE 2000

Date of mailing of the international search report
30 AUG 2000

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Form PCT/ISA/210 (second sheet) (July 1998)★